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THE HERTER LECTURES FOR 1916 GIVEN IN THE UNIVERSITY AND BELLEVUE MEDICAL SCHOOL, NEW YORK

BY

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ST. LOUIS

THE C. V. MOSBY COMPANY

1917

Press of
The C. V. Mosby Company
St. Louis

PREFACE

As stated on the title page this little book is made up of the Herter Lectures given in 1916 at the University and Bellevue Medical School, New York. These lectures were published in the Journal of Laboratory and Clinical Medicine. There has been some demand for reprints, of which none were made. I have, therefore, gladly acceded to the request of the publishers, The C. V. Mosby Company, who have offered to present them in the present form. The researches upon which they are founded have occupied much of my time for many years, and have given me a concept of the nature of infection, quite different from that usually taught.

Hoping that, in this form, my studies may be helpful to my fellow-workers in the profession, I submit this booklet to them.

THE AUTHOR.

Ann Arbor, Mich., 1917.

CONTENTS

INTRODUCTION

In nature all proteins are the products of life and each kind of living molecule elaborates and contains its own specific protein. Some forms of life are capable of constructing their proteins out of inorganic matter, while others can utilize only that which has been built up by other cells into protein material. Plants take the ammonia, nitrates and nitrites of the air, soil and water, and by synthetical processes convert these into the proteins found in their tissues. In this process there are two stages. In the first the inorganic nitrogen is synthesized into amino acids and in the second these are combined to form proteins. The higher animals cannot synthesize inorganic nitrogen into amino acids. This is done for them by plants and to some extent by bacteria in conjunction with plants. By the symbiotic action of certain bacteria and plants even the free nitrogen of the air is drawn upon in the construction of vegetable proteins. So far as protein metabolism is concerned the vegetable world is the synthetical or constructive labora-

tory while the animal is the analytical or destructive machine. The plant takes the smallest parts and builds them up into highly complex bodies, while the animal takes the complex and splits them into pieces to be reconstructed in its own body. In a general way the above statement is true, but there are synthetical processes going on normally in the animal body and it is demonstrable that simple proteins may be built into more complex molecules in the animal body. Moreover, it is certainly true that in man with perfect digestion practically all the nitrogen of the food is absorbed in the form of amino acids. The animal as well as the plant is a synthetical laboratory, but the new material used by the former is the finished product of the latter, which is unravelled and then woven into a new pattern which is different in each species of animal.

There are as many kinds of proteins as there are kinds of living matter. Chemically proteins are polymers of amino acids. The amino acids demonstrated in proteins are only about eighteen in number, but with these put together in an almost infinite variety of ways, we get an unlimited variety of products, just as with only twenty-six letters in the alphabet

there is no end to the making of words. The simplest proteins consist wholly of amino acids. These combine with inorganic salts, lime, phosphorus, iron, etc., and with carbohydrates to form the compound proteins.

All living things not only contain protein, but this is their essential constituent. The living protein molecule is in a labile or active state, capable of trading in energy, absorbing and eliminating; never in a condition of equilibrium. Dead protein is in a state of rest; it is a stabile molecule and remains in equilibrium.

POISONOUS PROTEINS

PART I.

BACTERIAL PROTEINS.

Material.—Fifteen years ago, after various attempts to secure bacterial cellular protein in large amount, I succeeded with the tanks for massive cultures which have been described elsewhere. The growths thus obtained are freed from extraneous matter by washing with dilute alcohol and then by thorough successive extractions with absolute alcohol and ether. The cellular substance is ground first in porcelain and then in agate mortars, and passed through fine meshed sieves. Whatever the bacterium employed the product is a fine white powder. The dilute alcohol removes the extraneous matter mechanically held by the growths and the long continued extractions with alcohol and ether remove coloring matters, fats, waxes, and other less known bodies. I have never made a close study of these extractives. These bacterial powders when examined microscopically show the individual cells plainly, es-

pecially when properly stained. Even the chromogenic bacteria come through as white powders, all the color being removed by the alcohol and ether. The freedom of this cellular material from extraneous matter is best appreciated from the fact that when one gram of it is incinerated there is no trace of chloride in the ash. With chloride of sodium in the culture medium and considering the ease with which traces of chloride are detected, this indicates a surprising degree of purity in the material. The significance of the absence of chloride will be discussed later. The cellular protein of many pathogenic and nonpathogenic bacteria has been obtained by growth in the tanks. I may say that I have never dared to grow anthrax bacilli in these massive cultures and have contented myself so far as this organism is concerned with the less abundant growths in Roux flasks. For obtaining abundant growths of the tubercle bacillus the tanks are less suitable than glycerine beef-tea cultures.

Chemistry.—It has been generally assumed that bacteria are unicellular plants. This assumption rests, so far as I can learn, upon early statements, such as that of Pollender, that anthrax bacilli are not affected by strong alkali and this has been interpreted as meaning that

they consist largely of cellulose. It is true that certain investigators have claimed to demonstrate even large amounts of cellulose in bacteria. Hammerschlag on wholly inadequate evidence estimated the per cent of cellulose in the tubercle bacillus as high as 28.1. DeSchweinitz and Dorset reduce this amount to 6.95 per cent, but hardly accept this figure themselves since they conclude that cellulose is probably present in small amount in the tubercle bacillus, and not present in the bacillus of glanders. These and other investigators, who have reported the presence of cellulose in bacterial cellular substances, have not properly distinguished between cellulose and other carbohydrates. Vincenzi employing proper tests failed to find cellulose in the cellular substance of bacillus subtillis, but did find a nitrogenous carbohydrate. In our work, Wheeler made special search for cellulose in sarcina lutea. Twenty grams of cell substance was autoclaved with 25 parts (500 c.c.) of ten per cent potassium hydroxide at 120°, first for thirty minutes and then for an hour. There remained a considerable residue which gave none of the protein reactions, did not reduce Fehling's solution even after prolonged boiling with dilute hydrochloric acid, but did respond to the carbohy-

drate test with alphanaphthol. Cellulose could not be detected by any known test. Schweitzer's reagent failed to dissolve it and it gave no color with iodine even after treatment with sulphuric acid. A portion was dried and heated with soda lime when it evolved a gas which turned red litmus paper blue, thus indicating the presence of nitrogen which had been reduced to ammonia. The odor of burning feathers also indicated the presence of nitrogen. From these results we conclude that there is no evidence of the presence of cellulose in bacterial cellular substance. Leach made search for cellulose in the cells of the colon bacillus with like negative results. Like Vincenzi we did find a nitrogenous carbohydrate. This is chitin or some chitin-like substance. The presence of chitin in bacterial cell substance has been reported by Ivanoff, Emmerling, Helbin, Bulloch, and others.

My students and I have found two carbohydrates in bacterial cellular substance. One, referred to above, is combined with nitrogen, is not soluble in strong alkali, and does not reduce Fehling's solution even after prolonged boiling with dilute mineral acids. The second carbohydrate is combined with phosphorus, is soluble in alkali, and does reduce Fehling's solution

after being boiled with dilute mineral acid. In the unbroken molecule this carbohydrate undoubtedly is contained within the nuclein group. If the presence of cellulose be essential to plant tissue, bacteria certainly are not forms of plant life.

There is no controversy concerning the presence of nuclein in bacterial cellular substance as the xanthine bases have been demonstrated among the disruption products both in my own laboratory and elsewhere. The literature on this subject is too extensive to permit me to go into it exhaustively and I will content myself with a few references. Klebs obtained from the turbercle bacillus a nuclein containing 8-9 per cent of phosphorus. From the same organism Ruppel separated a nuclein containing 9.42 per cent of phosphorus which he designated tuberculinic acid. Levin obtained from the tubercle bacillus proteins, nuclein, and crystals which he considered a mixture of thymil and uracil, also cystosin. Lustig and Galeotti obtained a nucleo-protein from the pest bacillus. In our work the presence of nuclein bodies was plainly in evidence. Leach obtained from the colon cell substance a body containing 7.33 per cent of phosphorus and both Leach and Wheeler

secured evidence of the presence of xanthine bases.

Bacterial cellular substance responds to all the protein reactions. Proteins are detached from the substance by both alkalis and acids, but the properties of the bodies thus obtained indicate that they are split products obtained by the cleavage of more complex molecules, and do not exist free in the cellular substance. Dilute mineral acid splits off the nitrogenous carbohydrate and when this extraction is carried on at high temperature much of the second carbohydrate is converted into a reducing substance. The acid extracts when dropped into a large volume of alcohol give a precipitate which after purification by resolution in water and reprecipitation with alcohol yields more than seven per cent of phosphorus. The line of cleavage through the large molecules in the cellular substance followed by acid action seems to be definite and the same products are obtained with one per cent and 0.1 per cent sulphuric acid. More concentrated acids after prolonged heating break deeper into the molecular structure and cleave the biuret bodies with the liberation of amino acids. Wheeler and Leach have made special studies of the action of mineral acids on bacterial cellular substance.

Ten per cent solutions of potassium hydroxide at 120° extract from bacterial cell substance everything except the chitin-like body which consists of a carbohydrate combined with nitrogen.

We have demonstrated the presence in bacterial cellular substance of both mono and diamino acids and have shown that the percentage of these varies with the microorganism. We have found the percentage of nitrogen to vary from 5.964 in subtilis to 11.765 in violaceous.

I began this work with the expectation of finding the bacterial cell substance composed of relatively simple bodies. I have been compelled to come to the opposite conclusion. The cellular substance of bacteria contains highly complex molecules. We have demonstrated the presence of the following groups among the split products: (a) A chitin-like body consisting of a carbohydrate combined with nitrogen. It seems reasonable to infer that this exists in the cellular substance as a glyco-protein. (b) A carbohydrate group combined with phosphorus from which it is not easily detached. This group reduces copper after prolonged boiling with dilute mineral acid. The amounts as determined by the reduction of Fehling's solution and calculated as xylose are large, but we are not sure

that the reducing substance is all carbohydrate. Indeed, it might be better to speak of both of these groups as those responding to the alphanaphthol test rather than as carbohydrates and to distinguish between them as non-reducing and reducing bodies. However, it seems clear that the one now under consideration is a subgroup in the nucleinic acid constituent of the cell substance. (c) The presence of nucleinic acid is beyond doubt, as is shown by the high phosphorus content of some of the split products and by the demonstration of the xanthine bases. (d) That one or more protein groups exist in the cell substance. If all these groups exist in the same molecule the cell substance must contain a highly complex molecule which would be best designated as a glyco-nucleo-protein. The fact that these bodies are removed only by agents capable of causing molecular disruption inclines me to the belief that the molecules which make the cell substance are highly complex. It may be said that this is an assumption and without adequate proof. On the other hand, such a statement as that made by Doerr, that bacterial proteins are of simple molecular structure, is wholly without evidence. Because bacteria are simple morphologically is no proof that they are made up of

simple proteins. This certainly is not true even if it should prove that I have overestimated the size of these protein molecules.

Tamura working in Kossel's laboratory has made a contribution to the chemistry of bacteria. He used cellular material obtained from the bacillus tuberculosis and mykobacterium lacticola perrugrosum. Both of these were grown in glycerine-broth cultures for five weeks, then collected on filters and washed with ether and alcohol. Tamura states that all the fats and waxes cannot be removed in this way and he resorted to the following method: After partial extraction with ether and alcohol the bacterial cells were rubbed up in a mortar with two parts of sulphuric acid and one of water and from this mass extraction with ether and alcohol was continued. From these extracts Tamura obtained along with the well known fatty acids diamino monophosphatide, a substance which has been previously found in egg-yolk, muscle, and brain. Tamura thinks that this body has been mistaken for lecithin by other investigators working with ether and alcohol extracts of bacteria. In these extracts Tamura has furthermore detected a higher alcohol, which he names "mykol" and to which the "acid fast" properties of these bacteria are

due. The statement that the fats and waxes including the phosphatide and "mykol" cannot be removed from the tubercle bacillus with alcohol and ether without previous disruption with strong acid is erroneous. In our work, first published in 1908, we showed that prolonged extraction of tubercle cell protein with alcohol and ether removed from the cells the substance to which the "acid-fast" property is due. We extracted first with alcohol in Soxhlets for four days and then with ether for three days. There is therefore no ground for the assumption by Tamura that either the phosphatide or the "mykol" is in chemical combination with the proteins of the cellular substance. It is much more reasonable to regard them as substances either on their way to assimilation into the cell molecules or as excretory products. Tamura says: "My investigations show that the presence of the diamino-phosphatide is not confined to the higher organisms." This is another assumption that the molecular structure of bacterial cells is simple and is wholly without justification. It is additional support of my contention that chemically the bacterial cell is highly complex and should not be regarded as a primitive form of life. Tamura's work on the cellular proteins strongly supports my claim

that these are highly complex in chemical structure. He states that he was not able to extract protein from the cell substance with water, salt solution, or one per cent sulphuric acid, and that even with alkali a portion of the cell substance remained undissolved. Surely this would not be true if the cell substance consisted of simple proteins. He also obtained the nuclein bases, diamino and mono-amino-acids. Of the last mentioned his list for the tubercle bacillus contains one (prolin) not found in ours, while ours contains two (glutamic acid and leucin) not found in his. Neither found glycocoll in the tubercle bacillus while we found it in the colon bacillus.

Tamura concludes, as we had done some years before, that bacterial cellular substance contains two carbohydrate groups, but the one which we have designated as chitin-like body, he classifies as a hemicellulose. This name was proposed by Schultze, after an investigation of various cell membranes, to include a group of bodies, "which are wholly soluble on being heated with dilute alkalis. In the cold, five per cent sodium hydroxide dissolves them somewhat more slowly." And yet Tamura classes as a hemicellulose a body which remains in the residue after repeated extraction with one per

cent sodium hydroxide; besides he did not test this residue for nitrogen but seems to have assumed that this element was not present since the body did not respond to the protein reactions. In our tests this body remained in the residue after heating for one hour at 120° with ten per cent potassium hydroxide, and did not give the protein reactions, but did contain nitrogen. We know of no nitrogenous carbohydrates except the chitins.

Tamura reports a negative test for sulphur in bacterial proteins, but this is due to faulty technic, since Wheeler has shown that the sulphur is masked and does not respond to the ordinary tests, but its presence is disclosed when a portion of the substance is fused with metallic sodium, dissolved in water and treated with a freshly prepared solution of sodium nitroprussiate; a violet color indicating the presence of sulphur.

While the "acid-fast" and "Gram-positive" properties of certain bacteria depend upon lipoids which are extracted from the cells by alcohol and ether, the cells, after exhaustive extraction with these solvents, take the analin dyes quite as well or even better than before. For instance, the extracted tubercle bacillus stains just as well, or even better, than before

extraction with alcohol and ether, but now the stain is easily removed by dilute acid. This behavior of bacterial cellular substance towards basic analin dyes quite naturally suggests that the former consists largely of nuclear material. In my opinion this is strengthened by the studies of the cellular substance which I have outlined. Additional evidence in the same direction is not wanting. When sporogenous bacteria form spores or pass into the resting stage the essential part of the bacterial cell is contained in the spores and all spores and reproductive cells consist in part at least of nuclear material. Certain bacteria which do not form spores pass into a granular state in which potential life continues for a long time. For instance, the bacillus of glanders, though an asporogenous organism, may retain viability for a long time. Wladmiroff states that he found these organisms in glycerine-bouillon tubes, with the ordinary cotton plug capable of growth after standing four years. The same is true of the plague bacillus. This phenomenon is explainable only on the assumption that these bacilli contain nuclein. However, assumption is no longer necessary since nuclein, nucleinic acid and their derivatives have been found in all bacterial cells submitted to chemical study. I

am strongly of the opinion that the bacterial cellular substance as I have prepared and studied it, freed from the extractives soluble in water, salt solution, alcohol and ether, is practically all nuclear material.

Some years ago A. B. Macallum by microchemical methods showed that nuclei are free from chlorine. His statement is as follows: "Intercellular material and structures, including the so-called cement substance of von Rechlinghausen are rich in chlorides but normal nuclei of animal and vegetable cells are absolutely free from them." When my coworkers found no chlorine in the ash of our cellular substance, I thought that this must be due to careless work. I could not believe that with the chlorides, especially sodium chloride, as abundantly distributed as they are, they could be wholly wanting in this material, but repeated examinations confirmed the first finding. The finding of chlorine would not have been a conclusive evidence that the material is not wholly nuclear, but the failure to find any trace of this element I regard as most convincing evidence that it is wholly nuclear. Furthermore, Macallum found that the phosphorus and iron in nuclear material are masked, that is, they cannot be detected without more or less marked disruption of the

molecule. This is true of our cellular substance. I may recall the further fact that the sulphur is so masked that even in the laboratory of so eminent a chemist as Kossel it was not detected in bacterial cellular substance. It is well known that in proteins sulphur exists in two forms, one being readily split off with dilute alkali forming a sulphide, while the other is obtained only when the disruption of the protein molecule is carried much further. The former is wanting and the latter present in our cellular substance. I do not suppose that all nuclear material has the same elemental constituents, indeed, it is not supposable that this is true, but the above facts seem worthy of consideration.

The laborious and valuable researches of Macallum have shown that nonnucleated organisms, such as cyanophycea, beggiota and yeast cells, contain nuclein, and this is probably true of every cell which is capable of reproduction. We are no longer quite willing to accept the dictum of Schultze, Hertwig and others that every cell must contain a morphologically recognizable part, known as a nucleus. We may insist upon the presence of nuclear matter, but not of nuclei. Some morphologists have seen the necessity of altering our conception of a cell. Bourne has proposed that

Schultze's definition be changed to read: "A cell is a corpuscle of protoplasm which contains a specialized element, nuclein."

It should be understood that the cellular substance which I have been discussing is not identical with that which exists in the living, multiplying bacteria. The latter consists of the former with the addition of all the extractives which I have removed by the solvents, such as water, dilute alcohol, absolute alcohol and ether. The living bacillus has been stripped of all its surrounding food supplies, its accumulated excretory products and its storehouse of fats, waxes, etc. I have a strong suspicion that in some of our bacterial reactions, notably with precipitins and agglutinins, these extractives are concerned, while the cellular constituents have no direct part. The active constituents of the culture, the agglutinable substance, is not, in my opinion, an essential constituent of the bacterial cells, but consists of one or more proteins closely associated with the bacterial cells. It may be a protein already split off from the surrounding pabulum preparatory to absorption and assimilation, or it may be an excretory product. My reasons may be stated as follows: (1) Agglutination does not destroy the viability or virulence of bacteria; therefore, the re-

action does not disrupt the living bacterial cell. (2) Thoroughly washed typhoid bacilli are not agglutinable. (3) When typhoid bacilli are thoroughly shaken in salt solution so as to remove their flagellæ and the bacilli are deposited in a centrifuge, the emulsion of flagellæ is agglutinable. (4) Neufeld has shown that when cholera bacilli are thoroughly cleansed by being shaken with one per cent alkali, which does not destroy them and only washes away adherent matter, they are not inagglutinable but produce no agglutinin when injected into animals.

Agglutination and precipitation are closely related phenomena. When a bacterial culture is filtered, some of the proteins about the cells pass into solution and constitute the precipitogen while some of the same class of near-cell proteins remain adherent to the cells and constitute the agglutinable substance or the agglutinogen.

I find that the bacterial cellular substances on standing undergo autolytic cleavage. We are just now examining a bottle of colon cellular substance which was prepared ten years ago. It was only air dried and contains a small amount of moisture. When freshly prepared water or salt solution extracted no protein, now

one-third the nitrogen passes into solution when the substance is treated with these solvents.

Poisonous Action.—We have found all the bacterial cellular proteins poisonous. Our earlier work was done with the colon cell substance. Since all of these bodies are insoluble in water or salt-solution, it has been necessary to administer them in suspension. Early studies demonstrated the following facts: (1) The poison is contained within the bacterial cell and does not under ordinary conditions diffuse into the culture medium.* It is true that old cultures may contain soluble poisons, but these result from autolysis. (2) The poison is not extracted from the cellular substance by water, saline solution, alcohol or ether, either at ordinary temperature or at the boiling point. (3) Heating, even to 140° in the autoclave does not destroy the poison. (4) Dilute (0.5 per cent) solutions of caustic alkali disrupt the cellular substance slowly and imperfectly. Stronger (2 per cent) solutions break up the cell substance and render the poisonous fraction soluble. (5) Boiling with dilute mineral acid (to 1 per cent) has but little effect.

At first we were much puzzled by the fact that smaller doses killed while larger ones failed to

*It is understood that we are speaking of cellular poisons and not of bacterial toxins.

do so. This was observed when we were administering the substance by intraperitoneal injection. Then we found that the more finely the substance was ground the smaller was the fatal dose. When the substance was only coarsely ground in a porcelain mortar and suspended in water it did not kill guinea-pigs on intraperitoneal injection in doses less than 1 to 40,000 parts body weight. When the same powder was more finely ground in an agate mortar, it killed 15 out of 16 animals at 1 to 75,000; 9 out of 28 at 1 to 100,000; 5 out of 8 at 1 to 200,000; 4 out of 34 at 1 to 2,000,000 body weight. We observed that when heavy suspensions were used, lumps of the substance remained undissolved in the peritoneal cavity after death or recovery. In these observations we found the solution of our puzzle. The poisonous action of the cellular substance is in proportion to the extent to which and the rapidity with which, it is split up by the secretions of the body cells and this cleavage is determined by the relative surface exposure of the substance to the action of the cleavage agents. I dare say that the difference in susceptibility as shown among the individual animals is due to the abundance and effectiveness of the secretions elaborated by the body cells.

As has been said, we found the cellular proteins of all the bacteria studied more or less harmful to animals when introduced parenterally. The size of the dose necessary to produce a fatal result varies greatly with the source of the protein. The cellular substance of bacteria to which in its living state an animal is highly susceptible does not kill that animal at all or does so only after large doses. We have injected into the abdominal cavities of guinea-pigs the cellular proteins of the tubercle bacillus in quantities of from five to two hundred mg. without causing death in a single instance, while on the other hand a fraction of a mg. of the protein from bacillus prodigiosus kills. To kill a guinea-pig one part of the cellular substance of the anthrax bacillus to 1,700 parts of body weight is necessary, while with the colon substance one part to 75,000 kills all animals provided the material is finely ground. In general it may be said that the more highly susceptible a given animal is to infection with a given bacterium the more difficult it is to kill that animal with the cellular protein of that bacterium. On the other hand, the more highly immune a given animal to infection with a given bacterium the more readily does that animal succumb to injections of the cellular proteins

of that bacterium. At first sight these statements seem wholly irrational, but when we study them we find that they are not only reasonable but in accord with what might have been reasonably predicted beforehand. The guinea-pig is highly susceptible to infection with the tubercle bacillus because the secretions of its body cells have no destructive action on that organism. This together with the fact that the bacillus tuberculosis can feed upon certain proteins in the guinea-pig's body are the essential factors in the susceptibility. The infecting bacillus finds an abundance of suitable food and meets with no resistance. On the other hand the guinea-pig is highly immune to infection with the bacillus prodigiosus because the animal's body cells supply secretions which are immediately destructive to this organism and the first of these bacilli finding their way into the animal's body meet with immediate and complete annihilation. But when the prodigiosus is grown in vitro and a sufficient amount of its cellular substance, dead or alive, is thrown into the abdominal cavity the same agency which has given the animal immunity to infection now causes it to fall a victim to the protein poison. These facts are of practical as well as scientific interest because they undoubtedly

form the basis of the frequently reported and well attested observations of some of the great clinicians of the past that the case mortality in certain infections, most notably in typhus fever, is much higher in the better nourished than in the less robust.

As I have indicated the cellular proteins when introduced parenterally into animals are not wholly harmless even when they do not kill. When the cellular substance of the bacillus tuberculosis is injected into the abdominal cavity of a guinea-pig it has no recognizable effect so far as the behavior or external condition of the animal shows. The dead bacilli are taken up in the folds of omentum and develop local tubercles. When the cellular substance of the colon bacillus is injected, a peritonitis results. In short, the lesions which follow infections result also from the injection of the dead cellular substance. I conclude from this that the lesions of the infections are not due to the activity of the living bacilli, but result from reaction between the bacterial proteins and the body cells.

Split Products.—In 1903, Wheeler and I found that the bacterial cellular proteins could be split into poisonous and nonpoisonous parts and later we showed that all true proteins can be broken up in the same way. This work has

been confirmed by many investigators. There are several ways in which this cleavage can be secured, but the most satisfactory is the one which we first employed. The dried protein, after exhaustive extraction with alcohol and ether, is repeatedly heated at 78° with a two per cent solution of sodium hydroxide in absolute alcohol. When this is done the poisonous fraction goes into solution while the nonpoisonous part remains undissolved and is removed by filtration. This is evidently a true cleavage and not a mere disintegration. The nonpoisonous portion contains all the carbohydrate and phosphorus of the original complex molecule.

The Protein Poison.—Since this body has been obtained from all true proteins, bacterial, vegetable and animal, so far examined, we have called it "the crude soluble poison;" "crude" because it is undoubtedly a mixture of chemical bodies and "soluble" in contradistinction to the bacterial cellular proteins from which it was first prepared. Aqueous solutions are somewhat opalescent, and may be quite turbid. Filtration through hard paper generally gives a clear filtrate but with some preparations we have found filtration through porcelain necessary to secure a perfectly clear solution. All the crude soluble poisons that we have obtained give the biuret

and Millon tests. None give the Molisch test, thus showing the absence of carbohydrate. Some give the Adamkiewicz and Liebermann tests while others do not. This test is believed to be due to the presence of tryptophane. The fact that the poisons from certain proteins do not respond to these tests indicates that Doerr's assumption that the poisonous action is due to the presence of this group is without support. The poison gives the Millon test most strikingly and in high dilution. This test is believed to indicate the presence of tyrosine and it is interesting to note that gelatine which contains no tyrosine does not yield the poison. Aqueous solutions are distinctly acid to litmus and this reaction is due to some organic body. Neutralization with alkalis and alkaline earths weaken the action of the poisons. Poisons from some proteins appear to form definite compounds with calcium and magnesium and at least some of the calcium bodies are inert. In the dry state the protein poison forms a brownish powder varying somewhat in shade with the protein from which it is obtained. All preparations have the same marked odor. It is much more freely soluble in absolute alcohol than in water. Whether it should be called a protein or not is a question. Proteins should not be solu-

ble in absolute alcohol. However this substance gives the biuret test and this is generally regarded as the most distinctive test for proteins. Its alcoholic solutions are precipitated by alcoholic solutions of copper, mercury and platinum. By means of these precipitants with subsequent removal of the metal with hydrogen sulphide, we have obtained our most potent preparations. By this method we have obtained a body which kills guinea-pigs of from two hundred to three hundred grams weight in doses of 0.5 mg. given intravenously. The poison is not an alkaloid, although it may be basic in character.

Action on Animals.—The comparative effects of the living bacillus, the dead cellular substance and the crude soluble poison on animals was first worked out by V. C. Vaughan, Jr. The organism used was the colon bacillus.

(a) *The Living Bacillus.*—When a guinea-pig receives a fatal dose of the living colon bacillus intraperitoneally there is a period of from five to twelve hours, varying with the size of the inoculation, during which there are no recognizable symptoms. We regard this as the period of incubation, and it is roughly proportional to the amount of the culture used and to some extent to the virulence of the organism or

the rate at which the bacillus multiples. This work was done with a bacillus, 1 c.c. of a twelve hour or older bouillon culture of which invariably killed within twenty-four hours. When this amount was given no effects became visible for a period of from ten to twelve hours. With larger doses the period of incubation was somewhat shorter, but with the largest doses of the richest cultures there is still a period of incubation. This measures the time necessary for two things to happen. First the bacillus must multiply sufficiently to supply enough poison to visibly affect the animal. Second, this poison must be made effective by being split out of the large molecule of which it is a part. Therefore, while the period of incubation is not accompanied by the development of symptoms which rise to the plane of observation, it is actually a critical period in every infection and the outcome depends upon whether the bacteria are all destroyed before a lethal dose of the poison has been developed by the multiplication of the bacillus and set free or made effective by the secretions of the body cells. It is during this period that natural and acquired immunity either save the day or, for the time at least, fail. In natural infection the number of bacilli introduced is small and in case of full immunity

these are all destroyed, there is no multiplication and the amount of poison set free in the destruction of the small number of the invaders is not sufficient to induce symptoms or to develop lesions. This is what happens when the smallpox virus finds its way into the body of one thoroughly immunized by a previous attack of the disease or by successful vaccination. When the immunity is only partial or when the infection is massive or unusually virulent, the virus develops for a time, becomes more or less distributed in certain tissues and its final destruction is accompanied by the development of symptoms, and the reaction between the virus and the body cells leaves more or less marked lesions. When there is no immunity the virus multiplies without hindrance and life is destroyed. There are infections in which the body shows little or no resistance. Some of these run an acute course and destroy life in a few days, while others are more chronic. This seems to depend upon the rate of multiplication in the invading organism. Apparently there is relatively as much difference in the rate of multiplication in bacteria as there is among the higher animals. The "generation period" or the interval between fissions varies among species and strains, and is influenced by external

conditions. Virulence is largely determined by rate of multiplication or at least the two correspond. Under favorable conditions the cholera bacillus divides about every half hour. So far as I know no one has determined the "generation period" in the tubercle bacillus, but it is certainly much longer. It follows that cholera is an acute disease, often terminating fatally in a few hours while tuberculosis extends through months and even years. The guinea-pig shows no resistance to the tubercle bacillus and the organism slowly but steadily grows, develops its characteristic lesions and kills, probably through its autolytic products and without developing any antagonistic action in the body cells. Rodents, especially rats, show but little or no resistance to the plague bacillus, except in those regions where this disease is endemic and there, it is said, this disease even among the rats becomes a chronic infection.

Our intraperitoneal infection of the guinea-pig is comparable with the development of a general peritonitis from a ruptured appendix. The period of incubation is short and while there may be some elevation of temperature, this is not marked or even constant. During the period of incubation, when the bacilli are abundantly multiplying, the behavior of the

animal in no way distinguishes it from its untreated fellows, but at the end of this period there is a marked change. The animal no longer eats; its coat becomes rough; its head droops; it sits in one corner of the cage in a stupor; its abdominal walls become rigid and pressure over this region elicits evidence of pain. Now, its temperature begins to fall and this decline is progressive in fatal cases. We have frequently seen the temperature fall from 101° to 94° in from two to four hours and it may reach 85° and even lower before death. A rise in temperature after it begins to fall generally means recovery. Autopsy reveals a general hemorrhagic peritonitis with a large amount of bloody fluid with intact red corpuscles and leucocytes in the peritoneal cavity. The parietal and visceral peritoneum are studded with minute punctiform hemorrhages and there is more abundant hemorrhage in the great omentum. The chemotactic pull of the bacilli has been not only great enough to assemble great numbers of leucocytes, but violent enough to rupture small blood vessels.

(b) *The Cellular Proteins.*—When a fatal quantity of the cellular protein of the colon bacillus is injected into the peritoneal cavity of a guinea-pig the progress of events is exactly

like that following infection with the living organism except that the period of incubation is shortened. There is no longer either opportunity or need for the multiplication of the bacillus. This has taken place in vitro and enough of the protein to kill has been introduced. One of the features that characterizes and marks the period of incubation has been withdrawn. It only remains for the body cells by means of their secretions to cleave the bacterial protein and set the poison free. The period of incubation is reduced half or more, then the evidences of poisonous action are exactly the same as in the inoculated animal. The temperature falls at the same rate and autopsy reveals exactly the same lesions. The chemotactic pull of the dead protein has proved just as strong and just as violent as that of the living protein. In fact the pull in both instances is a chemical and not a vital one and the lesions result from a reaction between the proteins of the bacterial cells and those of the body cells.

(c) *The Soluble Poison.*—When a fatal dose of the crude soluble poison is injected into the peritoneal cavity, the effects begin to reveal themselves much sooner. There is now no period of incubation. Both steps, which have characterized this period, are now omitted. The

bacillus has been grown and has been cleaved in vitro. The action of the poison begins to manifest itself within a few minutes—from five to twenty—and it appears in three well marked stages: The first we have designated as that of peripheral irritation. In the guinea-pig, it is manifest by the animal scratching itself, generally first on the nose and then over every part of the body which can be reached by its claws. In man, an erythematous blush, beginning about the point of injection, spreads over the body and may be followed by an urticarial rash with intense itching. This is not always confined to the cutaneous surface, but may extend to the mucous membrane of the mouth, throat and rectum. The second stage is one of partial paralysis. The guinea-pig lies on its side, with rapid, shallow and difficult breathing. When urged to move, it shows inability to coordinate its movements and partial paralysis is evident, especially in the posterior extremities. In man, the breathing becomes distressingly asthmatic. Air-hunger is marked and there is a sense of impending danger. The convulsive stage marks the termination. The convulsions are usually clonic and at first generally involve only the neck muscles, the head being thrown back. The seizures extend over the body, becoming more

frequent and violent. During a convulsion, occasionally in an interval, respiration ceases. The heart continues to beat, at first with no acceleration and with perfect regularity. The exact mode differs somewhat in different animals, but is always that of anaphylactic shock. Necropsy shows the same conditions found after death from anaphylactic shock. The peritonitis found after death from inoculation or from the injection of the unbroken cellular protein is wholly wanting.

When a nonfatal dose of the soluble poison is administered, the symptoms are those described above as characterizing the first and second stages. There may be isolated and slight convulsive seizures, but an animal seldom recovers after the convulsions have become general and frequent. With recovery the temperature slowly rises and ultimately returns to normal. Within two hours the animal is apparently quite normal in every respect.

PART II.

VEGETABLE PROTEINS.

Thanks to the researches of Osborne, a number of vegetable proteins may be obtained in a pure state and in quantity. The work done in my laboratory was upon some of the seed proteins, especially zein from cornmeal and edestin from hemp seed, which were prepared by Leach according to the methods of Osborne. From these proteins we split off the protein poison by the same process employed in the cleavage of the bacterial proteins. The poisons obtained from zein and edestin showed no difference either in response to chemical tests or in physiological action from those obtained from the cellular substance of bacteria. My present purpose in bringing out these facts lies in the evidence which they bear in support of my contention that the protein poison is a group in the protein molecule and that it is present in all true proteins. So long as my studies were confined to the highly complex bacterial proteins, I was not sure of the correctness of this idea. With edestin we are supposed to

have an unmixed protein. It is a single compound, of highly complex structure it is true, but not a mixture of different molecules. If the poison be detached from this by chemical cleavage it must follow that the poison consists of a group which exists within the larger body. The importance of this will be more evident when I call attention to the fact that some years ago Pick and Spiro were unable to obtain from edestin the substance which when injected into animals retards the coagulation of the blood and from this failure they concluded that this body is not a true cleavage product of proteins and that it is not an intramolecular constituent of pure proteins. In fact they came to the conclusion that the coagulation-retarding substance is neither a protein nor a protein derivative. The relation between the protein poison and the coagulation-retarding substance will be discussed later. At this point I simply wish to emphasize my claim that the protein poison is an intramolecular constituent of proteins and that it is obtained by the chemical cleavage of protein molecules. Edestin being a simpler and smaller molecule than bacterial cellular substance is the more suitable matrix from which the protein poison may be obtained. The yield is larger and the by-products less in va-

riety and abundance. Edestin contains no carbohydrate group while bacterial proteins contain two and these gave us much trouble in our earlier attempts to isolate the poison.

Animal Proteins.

We have prepared the protein poison from a great number and variety of animal proteins, such as egg-white, casein, serum albumin, serum globulin, blood cells, muscle, brain, liver, kidney, etc. In fact, we have found no true protein which does not yield the poison when split up by the method given—a two per cent solution of sodium or potassium hydroxide in absolute alcohol.

In beginning this work I expected to find the simplest proteins in unicellular organisms. As I have already indicated this expectation has not been realized. The proteins of most simple structure I have found in seeds and in the casein of milk. Seeds contain the embryo accompanied by simple proteins and varying amounts of fat and carbohydrate, also proteolytic, amylolytic and lipolytic ferments. When the seeds are placed under proper conditions of temperature and moisture, the ferments begin to act, the storehouses of foods are split into available building blocks and growth begins. In

milk the food supply for the young is supplied in similar form. The carbohydrate exists in the form of milk sugar. The fat exists as such. The protein, in the form of casein, supplies the amino acids and the mineral substances are found mostly in the ash. The ferments are furnished by the digestive organs of the young. Digestion is relatively simple and easy, absorption proceeds quickly and growth follows.

Bacteria, although unicellular and simple morphologically, are made up chemically of highly complex molecules. There may be unicellular organisms composed of simple proteins but this certainly is not true of the bacteria which I have studied. In their chemical composition and structure these bacteria are quite as complex as the most highly developed cells in the animal body. It follows, therefore, that when we speak of bacteria as low and primitive forms of life, we should bear in mind that we are speaking as morphologists and not as chemists. Many, probably all, of the soluble proteins in man's body are chemically of much simpler structure than are those of the bacterial cell.

The Proteoses.

Schmidt-Mulheim in trying to discover the fate of peptone in the blood (it being assumed

at that time that peptones are absorbed as such into the blood) found that the intravenous injection of Witte's peptone, after the removal of the undigested proteins from this commercial preparation, caused in dogs striking physiological effects. The most notable among these were: (1) an inhibiting action on the coagulation of the blood and (2) a rapid and marked reduction in blood pressure. This work done in Ludwig's laboratory was continued a year later by Fano. Furthermore, it was shown that a second injection of peptone made shortly after recovery from the effects of the first had but little effect. From these observations it became customary to speak of "peptone poisoning" and "peptone immunity." Fano did not confine his work to Witte's peptone, but made his own product by the digestion of fibrin with pepsin and trypsin. He also used Grüber's preparation and one from America. Grosjean used propeptone and peptone prepared by the method of Kühne and Chittenden and found that the former had a marked effect, especially when employed in doses of more than 0.15 g. per kilo. Arthus and Huber employed caseoses prepared by pancreatic digestion. Chittenden, Mendel and McDermott and later Chittenden, Mendel and Henderson produced highly poi-

sonous bodies by breaking up proteins with a vegetable ferment, papain, also with superheated steam and dilute acid without the aid of any ferment. Moreover, they found that all the primary, digestive protein derivatives have more or less marked effect upon blood coagulation and blood pressure. Pick and Spiro were unable to obtain a poisonous derivative from pure proteins, edestin and casein, and concluded that the poisonous agent present in mixed bodies is not a protein at all but an enzyme for which they proposed the name peptozyme. According to their view this in the proenzyme stage is widely distributed in the animal body, since they found the poison among the cleavage and digestive products of many organs. It might get mixed with the protein split products in digestion with an animal ferment, as pepsin or trypsin, or it exists in the tissue or protein which undergoes digestion; but take a pure protein like edestin or casein, and split it with acid and no poisonous body results. They claim that the poison never results from the hydrolysis of proteins with alkali. This is interesting in view of the fact that we have found cleavage with dilute alkali the best way of obtaining the protein poison.

Underhill has shown the incorrectness of the

claim of Pick and Spiro and demonstrated that the proteoses are in and of themselves poisonous, when administered intravenously. He prepared the poison from pure proteins by cleavage with acids and showed that native proteoses found in seeds and nuts, wheat embryo, hemp seed and Brazil nuts, when introduced into animals intravenously induce all the symptoms formerly known as those of peptone poisoning.

Popielski has worked with a body which he extracts from commercial peptone with alcohol. This "vasodilatin," as he calls it, has the same action that was formerly attributed to peptone and notwithstanding its solubility in alcohol it gives the protein color tests, at least the biuret and the Millon.

It must be evident that the behavior of my protein poison both chemically and physiologically, closely resembles that of the proteoses. Some proteoses, at least, are soluble in alcohol, and as has been said, Popielski extracts his body from commercial peptones with alcohol. The protein poison, though soluble in absolute alcohol, gives the protein reactions and is a biuret body; some proteoses behave in a similar manner. Edmunds has shown that the protein poison lowers blood pressure in dogs, just

as the "peptone poison" does. Edmunds did not find that the protein poison inhibits the coagulation of blood, but Underhill has recently showed that it has this effect, when used in larger doses than those employed by Edmunds. Underhill has recently compared the action of the protein poison with that of the proteoses and finds that the resemblance is strong both in the effect upon blood pressure and coagulation, but "Vaughan's preparation differs from the proteoses in that it produces marked symptoms or even death in the rabbit in relatively small doses." The rabbit is mentioned here because of its known refractoriness to proteoses.

It seems to me highly probable that the poisonous group in the proteoses is the protein poison and that its more powerful action is due to the fact that it has been more effectually stripped of those groups which tend to neutralize its effects. It is present in every true protein and when molecular disruption proceeds up to a certain point, the physiological action is increased, beyond that point it is decreased. The protein poison kills dogs, as shown by Underhill, in doses in which the proteoses have only a temporary effect, but the symptoms are the same. From this I conclude that the poisonous group is the same in both instances, but the free

poison is more effective than the combined. This belief is confirmed by the fact that the free poison is easily split out of the proteoses by proper chemical agents.

The Autolytic Cleavage of Proteins.

All proteins sooner or later undergo autolytic cleavage. When a solution or suspension of protein in water or salt solution is protected from bacterial invasion by chloroform or toluol and kept at about 37° the protein undergoes spontaneous cleavage. Salkowski seems to have been the first to investigate this phenomenon scientifically. This work has been continued by Biondi, Schwiening, Launoy, Jacobi, and others. Most of these have given attention to cellular autolysis, as this is the most interesting phase of the subject, but all proteins, whether cellular or without structure, go through a similar process. Fibrin undergoes autolysis quite as promptly as liver cells do. It is well known that in multicellular animals proteases are generally distributed. At first it was assumed that these consist of the alimentary ferments which have been absorbed and distributed through the body. However, research has shown that the autolytic ferments differ from either pepsin or trypsin. In the first place they

are possessed of a degree of specificity not characteristic of the alimentary enzymes. The ferment found in each organ or each kind of tissue digests especially, more rapidly and completely, the organ or tissue in which it is found. The liver ferment readily splits up liver tissue but is less effective in its action on the proteins of other organs. In the second place, the products of autolytic cleavage differ from those of enteral digestion. Pepsin forms large amounts of primary cleavage products, such as proteoses and peptones. These, especially the former, are highly poisonous, and would have a most disastrous effect were they liberated parenterally. The autolytic enzymes produce none or only traces of these primary split products. They cleave deeper and their chief products are the relatively harmless amino acids and purin bodies. From tryptic digestion the autolytic enzymes differ in several particulars. Trypsin acts in feebly alkaline solution while autolysis proceeds most rapidly in slightly acid media. It is more than probable that the intracellular tissue is always feebly acid. Tryptophan, a product of tryptic digestion, is seldom or never found among the autolytic products. In autolytic cleavage of proteins much more ammonia is found than in tryptic digestion. Further-

more the autolytic enzymes persist in animals from which the pancreas has been removed. We see from these facts that protein tissues disintegrate normally in the animal body without the formation of poisonous products. It must be admitted that in certain pathological conditions, such as acute yellow atrophy of the liver and in phosphorus poisoning, autolysis proceeds with harmful rapidity and becomes at least a highly destructive process.

It has been suggested that the autolytic enzymes are constituents of the blood and are generally distributed through the body by this fluid. In other words it has been held that they are blood ferments. That this is not true is shown by the fact that blood and blood serum have an inhibiting effect upon autolytic action. Besides, proteins which contain no blood, such as egg-white, undergo autolytic cleavage.

The study of autolytic cleavage is complicated by the presence in many proteins of other ferments such as nucleases, arginases, etc. What effects the autolytic enzymes have upon foreign proteins is a question of importance, but one which cannot be answered at present. It will be understood that I have been speaking so far of the autolytic enzymes of the cellular and other proteins of the multicellular ani-

mal. When we come to speak of the autolytic cleavage of unicellular organisms, such as bacteria, we have quite a different problem. That bacteria undergo autolytic cleavage and that the products formed in this process may be harmful to multicellular organisms has been abundantly shown. Old cultures of colon and typhoid bacilli may contain soluble split products which are highly harmful and indeed may be fatally effective in their action on the higher animals. Whether pathogenic bacteria undergo autolytic cleavage in the bodies of their hosts is a question which, so far as I know, has not been decisively determined by experiment. The presumption is that this may and does happen.

The following experimental data concerning the autolytic cleavage of bacterial proteins may be of interest in this connection:

Rosenow has shown that pneumococci suspended in salt solution and kept at 37° for forty-eight hours, under ether or over chloroform, undergo autolysis with the liberation of a poison. This poison injected intravenously or intracardiacly in normal animals induces anaphylactic shock. In guinea-pigs death results from bronchial spasm and consequent arrest of respiration. In dogs it causes marked fall in

blood pressure and delays the coagulation of the blood.

I took powdered pneumococcus cellular substance which had been prepared nearly seven years before. Microscopic examination showed the pneumococci as clearly defined and in as perfect form as in a fresh preparation. Five hundred milligrams of this powder was suspended in 500 c.c. of salt solution, 10 c.c. of chloroform added and kept at 37°. After twenty-four hours 10 c.c. of the opalescent supernant fluid was administered to a guinea-pig intravenously. Within two hours the animal's temperature fell to 94°, but recovery followed. The same experiment repeated after 48 and 72 hours killed the animal within two hours with the symptoms of subacute anaphylactic shock. A like injection after six days killed within three minutes with all the symptoms and post-mortem findings of acute anaphylactic shock.

It has been shown that the cholera bacillus does not undergo ready autolytic cleavage in vitro, but there is reason for suspecting that this happens in the intestine of infected men, since after death the bacillus is found only in the intestinal canal, in some instances at least, all the internal organs being sterile.

Warden finds that the gonococcus early un-

dergoes autolysis and that the autolysates are fatal to guinea-pigs. He believes that the autolysis of this organism is not due to enzyme, but results from a disruption caused by the absorption of water by the cells.

Parenteral Protein Digestion.

We now distinguish between enteral and parenteral digestion. We take diverse proteins into our alimentary canals and through the activity of the enteral digestive ferments they are split into amino acids which are utilized by the body cells in growth and in function. This is the normal way in which the body cells of the higher animals are fed, for the most part at least. Under normal conditions the amount of protein reaching the blood and lymph undigested is small and negligible in effect. Minute bits of unbroken protein may find their way into the circulation through the respiratory and digestive tracts. These, entering through the respiratory organs, may cause local sensitization which manifests itself in the complex of symptoms usually designated as hay—rose—or horse-fever and asthma. Those passing in undigested forms through the walls of the alimentary canal may lead to the untoward effects of certain articles of diet and possibly

may exert a more serious action on some of the more distant organs, especially the kidneys.

During fetal life all the food enters the body parenterally and there is no enteral digestion. There are reasons for suspecting that during infancy the chief milk protein, casein, may be absorbed in part in an unbroken state. At least in a few instances unchanged casein has been detected by the biological test in the blood of infants suffering from summer diarrhea.

In my opinion there are reasons for believing that in some animals a certain part, or certain kinds, of protein food are absorbed unbroken and are digested parenterally. Rabbits are easily sensitized, notably by casein fed by the mouth or administered by the rectum. I have detected the protein in the heart's blood by the biological test after such feedings. While there is a promising field for research along these lines, it is safe to say that in man in health, the amount of unbroken, foreign protein reaching the circulation is small. Protein in appreciable quantities reaches the blood only when injected, as in the employment of sera and vaccines or through infection. In the latter instance the protein multiplies in the body.

It is evident that one or more of the following effects may result from the parental intro-

duction of a foreign protein. (1) It may be eliminated through the kidneys. (2) It may be passed into the alimentary canal and there digested. (3) It may be digested parenterally. All these dispositions may be employed in the disposal of the foreign protein.

The literature concerning the renal elimination of foreign proteins is voluminous, but often contradictory. The occurrence and extent of this form of disposal vary with the kind of protein, the quantity, the rapidity of introduction, the species and individuality of the animal and probably upon many unknown conditions. It was formerly supposed that all the protein passing through the kidneys after parenteral introduction consists of that introduced. It has been definitely shown that this is not true and the estimates found in the older literature showing the per cent eliminated by the kidney are without value. Some years ago it was shown in my laboratory that in the urine of rabbits after the parenteral introduction of egg-white, both egg-white and blood protein appear. Guinea-pigs were sensitized to both with the urine. This gives no indication of the proportion in which they were present. It has been shown by Chiray and confirmed in my laboratory that foreign protein injected into the blood soon dis-

appears from the circulating fluid and carries with it an appreciable amount of the proteins of the blood. So far as I know Chiray is the only one who has made frequent observations of the effects of the parenteral administration of proteins in man. He frequently induced albuminuria in this way, especially in those who already showed renal inefficiency. In rabbits he induced marked structural changes in the kidneys by repeated injections.

In my work on the parenteral introduction of proteins, I have carefully controlled the rate of injection and have found that the foreign protein is more likely to appear in the urine when the rate of injecton is high. When the protein is slowly introduced, I have been surprised at the large amount that can be introduced into the abdominal cavity or into an ear vein without any detectable trace appearing in the urine.

When heterologous proteins are injected into the blood they soon find their way into the intestinal lumen. They are poured in with the bile and they pass into the abdominal cavity and through the intestinal walls. With the biological test we have detected proteins injected into the ear veins of rabbits in the liver, abdominal cavity and lumen of the intestines. It seems to be a general physiological law that poisons in-

troduced into the blood are eliminated in part at least into the alimentary canal. Morphine given subcutaneously may be detected in washings from the stomach. Gastric erosion may be induced by the subcutaneous or intravenous administration of arsenical preparations. So long ago as 1753 Sproegel showed that gastric lesions may be due to arsenic absorbed from wounds, and since that time they have been induced in animals by the hypodermic administration of neutral solutions of arsenic. Similar lesions are seen in poisoning with antimony and other metals and may result in these instances also from application made to wounds and to raw surfaces. Mercury when employed by inunction is poured into the alimentary canal and its destructive action may be seen in almost any part from the mouth to the rectum. Erosions of the stomach and intestine may be extensive and deep, even to perforation. The fact that gastric and duodenal ulcers may follow severe burns of the skin has been long known and is best explained by supposing them due to the large amount of poison resulting from the burn, being brought to the walls of the alimentary canal. The gastric inflammations and erosions of the acute infectious diseases are doubtlessly due to the same cause. The smallpox virus has

a predilection for epithelial tissues and manifests its destructive action in the skin and in mucous membranes. It has long been known that peptic ulcer is frequently associated with chronic appendicitis and the recent brilliant work of Rosenow has called attention to the probable relation between peptic ulcer and pyorrhea. In case of a nidus of infection in any part of the body poisonous proteins are being poured into the circulation and these like other poisons are carried to the walls of the intestine for the evident purpose of elimination. Here they accumulate and in their reaction with the body cells, the latter are more or less injured. The elimination of proteins from the blood into the alimentary canal holds for both living and dead, formed and unformed proteins. This is an interesting phase in the study of the action of poisonous proteins and is worthy of further study.

It has been long known that blood serum, like living cells, is highly resistant to proteolytic enzymes. Furthermore, the presence of blood serum markedly retards both peptic and pancreatic digestion. It has been generally inferred from these facts that blood serum contains an antiproteolytic ferment and since the reaction is alkaline, this is generally designated

as antitrypsin. So far as I know, Camus and Gley were the first to show experimentally that blood serum inhibits peptic and tryptic action. These investigators observed that fibrin or coagulated egg-white placed in serum and treated with active pepsin or trypsin remains intact. More extended observations have shown that many, if not all, kinds of proteolytic digestion, are retarded, often wholly arrested, by the presence of blood serum. There is another interesting fact in this connection. The injection of proteolytic ferments into an animal, especially repeated injections, increases the potency of the blood serum in the inhibition of the action of that ferment. Antibodies are formed and accumulate in the blood after repeated injections of pepsin, trypsin, rennin, etc. The effect of such injections is similar, probably closely related, to that which follows injections of toxins. But little is known concerning these antibodies in case of either the ferments or the toxin.

Delezenne and Pozerski first showed that chloroform removes from blood serum the antiproteolytic body. They found that blood serum has no digestive action on gelatin under ordinary conditions, but that blood serum which has been extracted with chloroform promptly digests gelatin. The researches of Jobling and

others have confirmed and amplified this work and it has been shown that when the unsaturated fatty acids are removed from blood serum by extraction with chloroform or ether, it becomes highly poisonous even for the species from which it was derived. Whether Jobling is right in his contention that the fatty acids constitute the antibody is still to be determined. It is possible that the extraction of blood serum with chloroform may have some effect upon the equilibrium in its protein constituents.

Friedberger found that the blood serum of the guinea-pig when incubated with bacterial cell substance becomes poisonous. He explained this on the assumption that the proteases of the serum digest the bacterial cells with the formation of a poison which he calls anaphylatoxin. Later it was shown that the guinea-pig serum when incubated with agar or starch becomes poisonous. From these findings it was suspected that bacillary substances, agar and starch, act upon guinea-pigs by absorption of the antibodies. In this way the proteases in the serum are relieved of the presence of their antibodies and digest the proteins in the serum. In other words, the matrix of the poison consists of the proteins in the serum and not of the bacillary cell substance.

Abderhalden found that when placental tissue is digested with the serum of pregnant women diffusible digestive products are formed and may be detected in the diffusate by the biuret and ninhydrin tests. He explained this by supposing that placental tissue in small amount finds its way into the maternal blood, and that this fluid acquires the property of digesting placental proteins. Abderhalden believes this to be a specific reaction and has proposed it as a diagnostic test for pregnancy. This test has been studied by many and while its significance cannot be considered as finally settled the weight of evidence seems to be that Abderhalden's explanation is not correct. It seems from the evidence now at hand that the placental tissue absorbs the antiferments and the unopposed protease of the serum digests the protein constituents of this fluid.

The weight of evidence today discards the idea of specific proteases in blood serum and favors the idea that certain antibodies exist in the serum and when these are reduced in amount, the nonspecific protease of the blood serum acts upon its own protein constituents. It must be admitted that this view is more in accordance with some of the facts than the one which holds that specific proteases are existent in the blood

or may be brought into existence. However, it should be stated that the present view does not exclude the necessity of regarding protein digestion in the blood as, in some instances at least, specific. Take the production of anaphylactic shock as an example. The theory proposed by Wheeler and me in 1907 supposes that when a given protein is first injected parenterally into an animal, it slowly develops a specific protease. This is a cellular product. Certain cells stimulated by contact and by penetration with the foreign protein develop a new, specific protease which is capable of digesting that protein and no other. The protein of the first injection is disposed of by this new specific ferment, but is broken up so slowly that no harm comes to the animal, or at least no recognizable danger, from the cleavage products. The cells continue in the possession of the newly acquired function. This may persist for years and indeed throughout life. The animal is said to be sensitized. On reinjection of the same protein the body cells, having acquired the function of digesting it, do so with such violence that the digestive products endanger the life of the animal or at least develop physiological disturbances which are easily recognizable. We have offered this in explanation of the success of vac-

cination. The vaccine virus is introduced into the child's arm. The proteins of which the virus is composed are distributed in the body and sensitize certain cells. This means that the cells develop a ferment which destroys the vaccine virus and the new function developed in these cells by their first experience with the smallpox protein in its attenuated form continues in the possession of the cells for years. When the vaccinated person is exposed to smallpox the virus of the disease is destroyed before it has time to multiply and consequently the individual is protected from the disease. Please understand that I am not ready to give up the theory of the formation of specific proteases. I see no other explanation of the immunity conferred by vaccination or by one attack of the disease. However, in presenting this matter I wish to proceed without being influenced by preconceived ideas, and I wish to repeat that the idea of a nonspecific protein digestion in anaphylactic shock especially has much in its favor, both in fact and in theory. The poison developed in anaphylactic shock may not come from the protein of the reinjection and the protease developed in sensitization may not be specific. Anaphylactic shock may be due wholly to the unmasking of a non-

specific ferment and the poison formed may come from the proteins of the blood, but if all this be true, and the weight of evidence today is in this direction, the anaphylactic reaction remains specific. We have only transferred the problem of specificity from the development of a specific enzyme to the specific uncovering of a nonspecific enzyme. It remains true that an animal sensitized to one protein is not sensitized to other and unlike proteins.

I have said that the theory of the uncovering of a general protease in anaphylactic shock has much in its favor. The blood seems to be a fluid in which ferments and antiferments are nicely and delicately balanced and a slight disturbance in this equilibrium leads to marked effect. We have obtained from one gram of casein enough of the protein poison to kill 800 guinea-pigs when injected intravenously. That casein, the chief protein constituent of the food of all mammalian young, should be found to contain a body so highly poisonous when introduced intravenously is certainly a surprising thing. However, the surprise does not disappear when we go further and find that a similar poison may be obtained not only from all the proteins we eat but also from those that make up the tissue of our own bodies. Indeed, every

gram of protein in an animal's body may supply enough poison to kill many such animals. There are other interesting things about this protein poison besides its potency. When amounts of it, even smaller than the minimum lethal dose, are incubated with blood serum in vitro, the serum, in itself inert, becomes fatally poisonous. In these studies a curious phenomenon has been observed. The incubating serum containing the poison may be fatally active at the expiration of a given time, then later wholly without effect, and later still fatally active. This wave of appearing, disappearing, reappearing toxicity we have frequently observed. For it, I have not even the shadow of an explanation. It may turn out after all that ferments and antiferments are not concerned in these phenomena. I have tried to think of oscillations induced in a colloidal fluid like the blood serum by the presence of the protein poison, but I have not been able to fix such a concept.

As was first shown by Friedberger bacterial cellular substance incubated with blood serum in vitro renders the serum poisonous. In repeating these experiments and injecting the serum at intervals, at one time it kills with all the violence of anaphylactic shock, then it has no effect, then again it kills. I have tried to time

this wave of toxicity, but adjust every condition to the best of my ability, I have been unable to chart it. It is to be hoped that some wiser man with more perfect control of the conditions of his experiments will solve this question. I am willing to leave it to those braver than I to try on human beings such poisonous mixtures of bacterial proteins as phylacogen.

I have stated that I am not yet ready to give up the idea that the parenteral introduction of foreign proteins produces specific alterations in the blood. I cannot do so, so long as I have the evidence supplied by the specificity of agglutination and precipitin reactions. We may have no proof that these are due to the development of specific proteases, but whatever their action it is within certain limits specific. Some years ago with my assistants I published the results of work which I interpreted as demonstrating the elaboration of specific proteases in sensitized animals. The results were all so clean cut and uniform that they were convincing to me at least. I will give a brief abstract: (1) One milligram of egg-white incubated at 37°, for thirty minutes in 5 c.c. of the serum or organ extract of unsensitized guinea-pigs is without effect when injected into the heart of another unsensitized guinea-pig. (2) Like re-

sults followed when the incubation was made with fluids obtained from an animal sensitized three days previously with egg-white. (3) When the fluids were obtained from animals sensitized fourteen days previously to egg-white, anaphylactic shock followed in all. (4) With the conditions as in (3) except that the incubation was prolonged to ninety minutes, the effects were less marked. (5) With the conditions the same as in (3) except that the incubation was done in a cold room the effects were nil. (6) When the fluids were obtained from an animal seventeen days after sensitization, anaphylactic shock resulted. (7) Filtration of the fluids through hard fiber paper did not affect the results. (8) Filtration of the serum and organ extract through a Berkefeld V did not affect the results. (9) Filtration after incubation did not affect the results. (10) When the serum and organ extracts were heated to 56° for thirty minutes there were no effects. (11) When the heated serum and organ extract were activated by the addition of unheated serum and extracts the effects were positive. (12) When the serum and organ extracts of a guinea-pig sensitized to egg-white were incubated with horse serum there were no effects. (13) The serum and organ extracts of guinea-

pigs sensitized to horse serum elaborated a poison when incubated with horse serum. (14) The serum and organ extracts of guinea-pigs sensitized to typhoid bacilli gave a poison when incubated with typhoid bacilli. (15) Like results were obtained with the cholera bacillus. (16) In case of egg-white the serum ceases to be active in about forty days after sensitization. (17) When the amount of protein incubated with the serum and organ extracts was larger than 1 mg. per 5 c.c. the results were less certain.

If there was not more luck than science in these experiments they clearly show specificity. I now know that there would be a chance of getting some positive results with a nonspecific serum, but it seems impossible for these results to have been so uniform on any other ground than that of specificity. Besides, they compare with the results obtained by Pfeiffer who found that the sera of guinea-pigs digested, for about forty days after sensitization, the protein to which the animal had been sensitized.

Abderhalden and his students in numerous experiments have shown by the polariscope that the blood serum of a sensitized animal has a more marked digestive action on the specific anaphylactogen than has the serum of a non-

sensitized animal. Similar results have been obtained by dialysis methods by Pfeiffer and Mita, by Pfeiffer and Jarisch, and by Zunz and György. The last mentioned have apparently shown a marked increase in amino acids during anaphylactic shock. This controverts the finding of Auer and Van Slyke.

I have repeatedly found, as others have, that the blood serum shows sensitization for relatively a short time, while the animal remains in a sensitized condition much longer. This observation has convinced me that protein sensitization is accompanied by and is due, in some instances at least, to a profound and lasting impression made on the cells of the body. Indeed, there can be no doubt that protein sensitization is cellular. Pearce and Eisenbrey bled a sensitized dog into a fresh one and at the same time replaced the blood taken from the sensitized one by that of a second fresh one. The sensitized dog from which all its blood had been removed responded with anaphylactic shock on reinjection, while the dog now carrying all the blood of the sensitized one did not.

PART III.

PROTEIN FEVER.

It has been known for a long time that the parenteral introduction of proteins in the animal body may be followed by fever. As early as 1883 Roques collected the literature of this subject and reported his own experimental studies. A few years later Gamaleia made a most important contribution to this subject. The title of this paper is significant and reads as follows: "The Destruction of Bacteria in the Febrile Organism." Gamaleia found that fever follows the parenteral introduction of bacterial protein, both pathogenic and nonpathogenic, both living and dead, consequently he concluded that fever is a result not directly of bacterial growth, but of bacterial destruction in the body. Indeed, he observed that attenuated bacteria often induce a higher and more persistent fever than the virulent forms. When a rabbit is inoculated with a virulent anthrax bacillus, fever develops but persists only a few hours, and then the temperature falls below the normal and death occurs. On the other hand,

when the second vaccine is used on a fresh animal, fever appears and continues for three days. When a highly virulent anthrax bacillus is employed there may be no fever and death follows within six or seven hours. Gamaleia made similar observations in other infections and came to the following conclusion: "Fever is not a result of bacterial growth, but on the contrary is consequent upon a reaction on the part of the body against the bacteria and leads to their destruction." Furthermore he found that nonpathogenic bacteria, living or dead, led to the development of fever. I think that these experiments, made more than a quarter of a century ago, furnish strong support of my theory that fever is due to the parenteral destruction of proteins. One year later this work was confirmed by Charrin and Ruffer and was shown to hold good for nonbacterial proteins as well. In 1890 Buchner induced the characteristic phenomena of inflammation—calor, rubor, tumor, and dolor—by the subcutaneous injection of diverse bacterial proteins. Krehl and Matthes induced fever by the parenteral administration of albumoses and peptones, but did not obtain constant and uniform results, because as we now know they did not recognize the necessity of regulating the size and fre-

quency of the doses. In 1909 my students and I showed that by regulating the amount and frequency of the dosage we could induce any desired form of fever, acute, fatal, intermittent, remittent or continued.

Inasmuch as I have given elsewhere* the details of this work I will only reproduce the conclusions and make a few general remarks: (1) Large doses of unbroken protein administered intraabdominally, subcutaneously or intravenously have no effect on temperature, at least do not cause fever. (2) Small doses, especially when repeated, cause fever, the forms of which may be varied at will by changing the size and frequency of the dosage. (3) The effect of protein injections on the temperature is more prompt and marked in sensitized than in fresh animals. (4) The intravenous injection of laked blood corpuscles from either man or the rabbit causes in the latter, even in small quantity, either in single or repeated doses, prompt and marked elevation of temperature. (5) Laked corpuscles after removal of the stroma by filtration have a like effect. (6) Protein fever can be continued for weeks by repeated injections, giving a curve which cannot be distinguished from that of typhoid fever. (7) Pro-

*Protein Split Products in Relation to Immunity and Disease, Lea & Febiger, 1913.

tein fever is accompanied by increased nitrogen elimination and gradual wasting. (8) Protein fever includes most instances of clinical fever. (9) Animals killed by experimentally induced fever may die at the height of the fever, but as a rule the temperature falls rapidly before death. (10) Fever induced by repeated injections of bacterial proteins and ending in recovery may be followed by immunity. (11) The serum of animals in which protein fever has been induced digests the homologous protein in vitro. In view of recent work on antiferment in blood serum this point needs reinvestigation. (12) Fever is one of the results of the parenteral digestion of proteins. (13) There are two kinds of parenteral proteolytic enzymes, one specific and the other nonspecific. (14) The production or activation of the nonspecific ferment is easily and quickly stimulated. (15) The development of the specific ferment requires a longer time. (16) Sensitization and lytic immunity are different manifestations of the same process. (17) Foreign proteins, living or dead, formed or in solution, when introduced into the blood soon diffuse through the tissues and sensitize the cells. Different proteins have predilection places in which they are deposited and where they are, in large part at least, digested, thus

giving rise to the characteristic symptoms and lesions of the different diseases. (18) The subnormal temperature which may occur in the course of a fever or at its termination is due to the rapid liberation of the protein poison which in small doses causes an elevation, and in larger doses a depression of temperature. (19) Fever per se must be regarded as a beneficent phenomenon inasmuch as it results from a process inaugurated by the body cells for the purpose of ridding the body of foreign substances. (20) The evident sources of excessive heat production in fever are the following: (a) that arising from the unusual activity of the cells supplying the enzyme; (b) that arising from the cleavage of the foreign protein; (c) that arising from the destructive reaction between the split products from the foreign protein and the proteins of the body.

The above are the conclusions which I drew three years ago from experiments which my students and I had carried out and from a study of the literature of the subject. I did not suppose at the time, nor do I hold now, that all these conclusions are exactly right.

The fundamental fact that the parenteral introduction of proteins may induce fever is founded upon so many independent observa-

tions, some of them recorded many years ago, that I do not think it incumbent upon me to seek additional support. Friedberger has, in a most exact way, confirmed the statement that large doses of foreign protein do not, while small doses do elevate the temperature. Moreover, he has shown that a small dose is more effective in sensitized than in unsensitized animals.

Thiele and Embleton have confirmed experimentally the proposition that the parenteral introduction of foreign proteins affects the temperature, causing a rise or fall or having no effect according to the size of the dose. They give the following tables:

EGG-WHITE.

Limits of	NORMAL ANIMAL grams	SENSITIZED grams
Temperature fall.....	0.05	0.005
Constant temperature....	0.02	0.0002 to 0.0001
Temperature rise.....	0.01 to 0.001	0.0001 to 0.000002

TUBERCLE EMULSION.

Limits of	NORMAL ANIMAL grams	SENSITIZED grams
Temperature fall....	0.005 to 0.002	0.0005
Constant temperature.	0.002 to 0.001	0.0001
Temperature rise....	0.001 to 0.00001	0.00001 to 0.000001

Criticism of the statement that foreign proteins find certain predilection tissues in which they accumulate has been made. Iodine ac-

cumulates in the thyroid gland. Mercury induces characteristic lesions in the kidneys. Strychnia selects a definite portion of the nervous tissue on which its action is made manifest. The therapeutic effects of the most approved drugs depend upon their predilection for certain tissues. The recent studies of Rosenow indicate that bacterial proteins do not differ from other poisons in this respect. We are accustomed to think of chemotaxis as acting only between morphologically recognizable bodies, but in reality it is a form of chemism and is dependent upon chemical composition and not on histological structure.

The only one of the above given statements formulated some years ago which has met with any experimental negation is my contention that specific proteases are developed by the parenteral introduction of foreign proteins. I am ready to admit that Friedberger's anaphylatoxin comes from the serum. In fact at the same time that I formulated the proposition concerning protein fever I wrote as follows: "It has been suggested: (a) That the agar or kaolin or bacteria absorb the complement from the serum and that this renders it poisonous. (b) That the poison is preformed in the serum, but that its action is neutralized by some other

constituent of the serum which is absorbed by the agar or kaolin. (c) That the absorption of some constituent of the serum by the agar, kaolin or bacteria leads to a disturbance of the equilibrium of the protein constituents of the serum which as a consequence break up with the liberation of the poison. These suggestions assume that the poison comes from the serum and this may be true." On another page I said: "That the anaphylatoxin comes from the blood serum, the one constant factor in all the experiments in its production, is most probable." Now since the probability has become a certainty, we need not conclude that specific proteases never result from the parenteral introduction of proteins. I have shown that all proteins, including those of blood serum, contain a poison and I am not at all surprised on learning that such a poison in the serum is set free in the production of Friedberger's anaphylatoxin and in the development of Abderhalden's pregnancy test, but these have nothing to do with the development of proteases in smallpox or typhoid fever. At least no such connection has been shown.

The Phenomena of Infection.

I have elsewhere gone into some detail concerning the views of the nature of infection

which I have developed in my studies on the chemistry and toxicology of bacterial and other proteins. Only a living thing can infect. Injection of diphtheria or tetanus toxin may cause all the symptoms and lesions of the respective diseases, but such injections are artificial procedures and the results are intoxications rather than infections. In this section I shall omit diseases due to toxins. The infecting agent is a virus and in infections there is a contest between the invader and the native. It is a struggle for food, growth, and reproduction. In the bacterial diseases the structure or the equipment of the invader is quite as complicated and as complete as that of the defender. The contest is between bacterial and body cells and the battlefield may involve only a small part or may extend to every part of the animal's body.

What is the difference between pathogenic and nonpathogenic bacteria? In order for a given bacterium to be pathogenic to a given animal it must be possible for the former to feed upon the latter. All living things feed by means of digestive ferments. Continued life and multiplication are impossible under other conditions. First, in order for a given bacterium to infect a given animal the ferments of the

former must be able to digest the proteins of the animal. In the second place the invading cells must not be immediately destroyed by the ferments elaborated by the body cells. There must be a supporting relation between the bacterial cell and the medium, and in infection the body constitutes the medium in which the bacteria grow and multiply. The protein groups split from the medium must fit into the molecular structure of the bacterial cell; otherwise they would be of no service to it. Many kinds of cells may live in the same medium, but for each kind the cleavage of the medium must be specific. From this it follows that the agent by which the cleavage products are secured must be supplied by the cell and must be specific to it.

It follows from what has been said that a bacterium placed in a medium in which its ferment is ineffective cannot grow and multiply. A bacterium which cannot grow and multiply in the animal body cannot cause an infection. Its inability to grow and multiply in the animal body may be due to the fact that its ferments cannot digest or properly break up the proteins of the animal body. This is one of the reasons why the great majority of bacteria are harmless or nonpathogenic. This, however, is not the sole, and probably not the dominant cause

of the failure of so many species of bacteria to do harm to the higher animals. What has been said about the production and utilization of ferments by the bacterial cell is equally true of the body cell. In fact, it is true of every living cell. The body cell has its specific ferments, and the bacterial cell being protein substance is liable to be digested by the ferments elaborated by the body cells. In these simple facts lies the fundamental explanation of all forms of bacterial immunity, either natural or acquired. It will be understood that I am here omitting all reference to the elaboration of toxins and antitoxins.

Ferments are intra- and extracellular. All are formed within the cell, but some diffuse into the medium while others do not. In some instances at least, cell permeation by the pabulum is essential to the feeding of the cell. In other cases the ferment accumulates on the surface where digestion proceeds. In others the ferment diffuses into the medium more or less widely from the cell which produces it. Many cells produce both intra- and extracellular ferments, and these differ in function.

I am not going into detail concerning cellular ferments. Those of the bacterial cells are easily obtained and have been studied quite elabo-

rately. Some digest proteins, such as gelatine, quickly while others are less prompt and others still have no recognizable effect on this protein. They are easily affected by the presence of certain nonprotein substances, especially carbohydrates. The ferments of the body cells are not so easily obtained and are more difficult of study. However, both the intra- and extracellular ferments of the polymorphonuclear corpuscles have been studied in some detail and their destructive action on certain bacteria has been demonstrated. The germicidal action of the blood and its serum has been demonstrated on various species of bacteria.

It may be well to point out some differences between intra- and extracellular ferments. The latter are comparable to the enzymes of the alimentary canal. Their function is solely a lytic one. They break up complex proteins into simpler bodies, but these without further treatment are not ready to be built into the cellular structure. The extracellular ferments are in a general way destructive in action. The intracellular ferments are essentially constructive. They shape the rough blocks and fit them into the molecular structure. In the process of infection the intracellular ferments of the bacterial cells are most active. The soluble, simple

proteins of the fluids of the animal's body are quickly built into the bacterial cell and growth and multiplication result. Body proteins are converted into bacterial proteins. This process proceeds so smoothly that as a rule during the time when its development is most rapid the host is quite unaware of the presence of his undesired guest. Whole molecules of albumins and globulins are taken into the bacteria and built into the more complicated bacterial cell. This is the period of incubation in an infection. The body cells are not prepared to combat the invader during this period. Finally the body cells react and begin the elaboration of ferments which destroy the bacterial proteins. This is quite a different process. Complex, cellular proteins are split into simpler ones and protein poisons are set free.

During the period of incubation of an infectious disease, the infecting organism supplies the ferment, the simple, soluble proteins of the body fluids constitute the substrate, the process is essentially constructive, no poison is set free and there are no recognizable clinical symptoms. During the active progress of an infectious disease, the body cells supply the ferment, the complex, bacterial, cellular proteins constitute the substrate, the process is essentially de-

structive, the protein poison is set free, the symptoms of disease appear, lesions more or less destructive develop and life is placed in jeopardy.

The experienced clinician will easily understand that in most infectious diseases the steps in the evolution of the processes are not so clearly defined as indicated in the above statements. They are most typical in uncomplicated cases of yellow fever, typhoid and typhus and in smallpox, but even in these there often are complicating factors. In yellow fever an attempt is made to eliminate the poison into the alimentary canal as is evidenced by black vomit. In typhoid the poison in being excreted into the intestine may lead to perforation. In most infections, the bacterial growth and their disruption overlap. In one part of the body the bacteria continue to grow while in other parts they are being destroyed. In pneumonia life may be endangered by the abundance and extent of the exudate, while in the crisis of this disease autolysis probably plays an important role not only in the destruction of the organisms, but in the removal of the exudate. In many infections lesions develop and impair the efficiency of the body cells. Moreover in destructive lesions the dead tissues of the body must be disposed of

and this throws an increased burden on the body cells. In some diseases phagocytosis plays an important role. It must be evident that the engulfment of bacteria by phagocytes is a more conservative method of disposing of the invading cells than their extracellular destruction, since in the former the body is protected against the poison liberated by bacterial cleavage. Nothing more dangerous to the infected individual could happen than the sudden cleavage of all the bacteria in his body. The poison liberated in this process would overwhelm him at once. This is a probable explanation of the fact, already referred to, that the case mortality in typhus fever is higher among the well nourished than among the less robust. Bacterial cells, as well as body cells, have means of protecting themselves. The tubercle bacillus through limitless generations of parasitism has developed coatings of fats and waxes which protect it against the action of secretions of body cells quite as efficiently as coats of mail protected our ancestors against the weapons of their time. Moreover, bacterial cells may develop increased resistance or become to some extent immune to the action of body cell secretions. Occasionally bacteria persist in the body for long periods after recovery

from the disease and when these are transferred to new hosts they show that they have lost nothing in virulence. Frequently, secondary infections develop and decide the fate of the individual. As someone has said the pyogenic microorganisms frequently play the last act in the great tragedies of life, tuberculosis, cancer, and syphilis.

A Chemico-Biologic Concept of the Protein Molecule.

Under this heading I wish to formulate certain theories which have developed in my mind during the progress of the work which I have outlined in preceding lectures. Some men seem able to work without developing theories and probably this is best, but I have never worked in that way. It is possibly a fault; if it be, I am ready to confess that I have sinned and continue in the same old way. I hope that some of the statements which I am about to make will stimulate others to investigate and this I deem of more importance than their truth or falsity.

The protein poison about which all my work has centered is a fact. It has been prepared and studied by so many competent men that its wide distribution in proteins from diverse

sources cannot be questioned. Its effects on animals have been widely tested and the general conclusions reached are quite as uniform as those which might be formulated about poisons much longer known. Its chemical structure has not been determined with certainty. The best evidence at hand today seems to indicate that it is not a basic body, and therefore not a protein alkaloid, not a leucomain or a ptomain. It contains no phosphorus and no carbohydrate. In the purest form in which it has been obtained, it yields a trace of ash of which phosphorus and chlorine are not essential constituents. Whether this mineral matter is an essential part of the poison or not, I do not know. Under any condition in which it has been obtained it is decidedly acid in character and yields amino acids on disruption. It seems to be a polypeptid.

Underhill, whose opinion I esteem highly, concludes that the action of the protein poison on animals is similar in kind but more intense than that of proteoses. I dare say that this is quite right and it conforms with my own observations. I suggested in the Shattuck lectures in 1906 that the protein poison is the chemical nucleus, keystone, or archon of larger and more complicated protein molecules.

The chemism of the protein poison is intense and it combines with various inorganic and organic substances to form more complex molecules, still retaining and imparting to these larger molecules its protein characteristics. Combined with phosphate of lime it forms such phosphoglobulins, so-called, as casein. Combined with carbohydrate it develops the glycoproteins and in combination with both phosphorus and carbohydrates, the glyco-nucleoproteins result. In the last mentioned bodies the protein molecule reaches its most complex form, and further development is possible only by polymerization and the aggregation of many protein molecules into cells. At what stage in the evolution of the protein molecule metabolism begins I cannot say, but it is quite evident that multiplication does not begin until the most complex structure has been reached. It seems quite evident that from the beginning the process is a synthetical one.

It is possible to conceive of the beginning of life on the earth, as proceeding in this way. In the intense heat of past geological ages when even carbon existed in the gaseous state this element combined with nitrogen forming cyanogen. With this binary compound under proper conditions the synthesis of the simplest amino

acid was possible for cyanogen may react with boiling hydroiodic acid with the development of amino acetic acid and from this the other amino acids found in the protein molecule might have been developed. In this view, proteins in their simplest form may have come into existence long before life as we now know it was possible on the earth.

The simplest protein, as the protein poison, has its intense chemism satisfied as it combines with other elemental groups in the development of the more complex bodies.

I began my work with the hope of finding simple proteins in the cellular structures of bacteria. In this I was disappointed and I now see that I should not have expected it. Instead of finding simple proteins in bacterial cells I have found them in the casein of milk and in the proteoses of seeds. As I have already said the young mammalian is fed upon food principles served in the simplest form. The nursing child is supplied with fats as such, with mineral constituents for the most part uncombined, with carbohydrates in the easily assimilable form of lactose and with amino acids in the relatively simple protein, casein. The sprouting seed finds the amino acids with which it starts life in the relatively simple proteins while fats

and carbohydrates are supplied in a ready-made form. Now if this provision be made for the support of the developing plant and animal, what can be said about the food supplied the numerous cells of the body, whether it be plant or animal. Simple proteins exist in the circulating blood of the higher animals. Not only is this true but as Van Slyke and his co-workers have shown the body cells directly use amino acids. The simple proteins probably exist in the circulating blood chiefly in that protein mixture about which we know but little and which we designate as serum globulin. In this mixture the primitive proteins are ready to enter into combination with the more complex cellular proteins as the latter wear away in their functional activities. Their chemism is held in abeyance by combination with some indifferent substances, such as calcium. I have found that the protein poison from casein is neutralized in vitro by calcium lactate. Indeed the protein poison is largely, but not so quickly, neutralized by incubation with sodium bicarbonate. In this connection it may be well to recall the effect of the withdrawal of calcium on the coagulation of blood and that after severe poisoning with the protein body the clotting of the blood is retarded and often wholly prevented. If my idea

that the circulating blood at all times contains the protein poison from the too violent chemism of which the body is normally protected by its combination with an inert body is correct, it will not be difficult to understand that the equilibrium may be disturbed in a variety of ways with death as a result. The introduction of a little more of the poison or the removal of the protecting body may seriously upset the equilibrium. Casein yields about ninety per cent of its weight in protein poison. The calcium is easily removed from casein. An ash-free casein may be prepared by repeated solution in dilute ammonia and reprecipitation with dilute acid. The last trace of calcium is removed by treatment with oxalic acid. The protein poison from casein resembles the globulins inasmuch as it may be wholly precipitated from aqueous solution by saturation with sodium chloride, but differs from globulins inasmuch as it is freely soluble in absolute alcohol.

Blood is rendered poisonous not only by incubation with bacteria, agar, starch, kaolin, etc., but as was shown by Köhler as long ago as 1877, it becomes poisonous on clotting, killing both homologous and heterologous animals. This phenomenon, which has been confirmed by others, has recently been investigated by Mol-

dovan who has shown that blood freshly defibrinated by shaking with glass beads causes acute death when injected intravenously into guinea-pigs and rabbits. In the former the typical anaphylactic lung picture is seen after death. When the dose is slightly sublethal there is marked fall in temperature with subsequent fever. When the doses are smaller there is marked fever. On standing from fifteen to forty-five minutes defibrinated blood loses its toxicity. Serum obtained by rapid centrifugation of defibrinated blood is poisonous. The same is true of the deposited and once washed corpuscles. When coagulation is delayed by the presence of sodium citrate neither the supernatant fluid nor the corpuscles are poisonous, but both become so when coagulation is induced by shaking with porcelain beads. Doerr has shown that blood received in paraffined vessels becomes poisonous; but when the coagulation is complete the toxicity disappears. When coagulation is made to proceed slowly by the addition of hirudin solution or a 0.7 per cent solution of colloidal silicic acid, it retains its toxicity for several hours.

The fact that extracts of normal tissue, when injected intravenously, are poisonous is another interesting fact. If the lungs of a rabbit be

macerated for two hours in salt solution, the solution kills promptly on intravenous injection. Homologous organ extracts are more poisonous than heterologous.

All these phenomena show that there is under normal conditions a nice adjustment in the constituents of blood and tissue whereby life is protected and that slight changes easily disturb this equilibrium with most disastrous results. There are here unsolved problems but my work leads me to the conclusions that there are protein bodies in the blood and tissue, which serve under normal conditions as cell foods, but which may become explosively poisonous when the mechanism regulating their use is disturbed. Normal cells contain deposits of these bodies, which under proper regulation, supply cell waste, but under abnormal conditions lead to cell destruction. These substances were probably present in my bacterial cells, but I washed them out and threw them away leaving only the cellular proteins. However, time and labor will solve these problems and I turn to another phase of my subject.

If I properly interpret my work on the chemistry of bacterial proteins it confirms the theoretical views of Pflüger, Ehrlich, and Verworn, who have held that the essential part of

cells consists of a chemical unity, made up of giant molecules. So far as I can find, this view receives additional support in the experimental work done by others. I have been able to find but little upon this subject. Reinke and Rodewald found that air dried substance of æthylium septicum, which they designate as plasmodium, consists largely of highly complex proteins containing phosphorus and yielding xanthine bases and carbohydrates on disruption. Sosnowski concludes from his study of infusorial cellular substance that this does not contain simple proteins as such, but as constituents of highly complex molecules. My studies have led me to formulate a theory concerning the nature and operation of living matter. My first attempt in this direction was made in a lecture delivered in Toronto (1905), and this was elaborated in a Shattuck lecture (1906). The cell is not the unit of life; life is molecular. Life is function, not form. The cell is not only made up of protein molecules, but its form and function are determined by the chemical structure of its constituent molecules. The lines along which the spore, seed or ovum develop are determined by the chemical structure of its proteins. Growth in other directions is impossible, and this accounts for stability in repro-

duction. However, changes in the chemical structure may and do occur and in these lies the basis of variation.

The keystone or archon of the protein molecule is the protein poison. It is common to all protein molecules. Physiologically it is the same in all molecules; i. e., when set free it is a poison and it is a poison on account of its intense chemism which enables it to tear off groups from other proteins. One protein differs from another in its secondary and tertiary groups. Most native proteins are not poisonous because in them the chemism of the primary group is satisfied by combination with secondary groups. Strip off the secondary groups and the primary becomes poisonous on account of the avidity with which they combine with the secondary groups of other molecules. Biological relationship between proteins depends upon the secondary groups. In this way varieties and species have developed.

The living molecule is never in a state of equilibrium. There is a constant exchange of atoms between it and the outside world. It absorbs, assimilates and eliminates. It is constantly trading in energy. It takes in oxygen and gives off carbonic acid; it takes in nitrogenous material, and, having utilized it, the waste

is discarded. The living molecule passes through the period of growth and decay. During the former, its functions are largely synthetic; in the latter they are autolytic and finally the structure drops into pieces.

PART IV.

THE PURITY OF ALCOHOL.

In my work with the protein poison, I have found it necessary to give attention to the purity of the alcohol used and I have found the alcohol imported from Germany (Kahlbaum's) not always up to the standard. In fact, I have found it cheaper and safer to take the ordinary alcohol and distill it with quick lime. The following tests may be used in determining the *purity of alcohol.*

1. The specific gravity is determined by the picknometer.

2. It should be miscible in all proportions without cloudiness with water, ether and chloroform.

3. It should not redden litmus even after four hours' exposure. Kahlbaum's alcohol often reddens litmus in a much shorter time.

4. The evaporation of 50 c.c. should leave no weighable residue. Some samples of Kahlbaum's alcohol gave in this amount as much as 1 mg. and some more.

5. A mixture of 10 c.c. of the alcohol, 5 c.c.

of water and 1 c.c. of glycerine, allowed to evaporate spontaneously on clean blotting paper, leaves no foreign odor when the last trace of the alcohol has disappeared. This is a U. S. P. test and shows the absence of more than a trace of fusel oil.

6. A mixture of 10 c.c. of the alcohol and 0.2 c.c. of two per cent KOH solution is evaporated to 1 c.c. and then treated with an excess of dilute (1:4) sulphuric acid. This should not develop the odor of fusel oil.

7. 10 c.c. of the alcohol is evaporated to 2 c.c. and this is shaken with an equal volume of sulphuric acid. The development of a reddish color shows the presence of amylic alcohol.

8. When 20 c.c. of the alcohol is shaken in a clean glass stoppered bottle with 1 c.c. of silver nitrate, test solution, the mixture should not become more than faintly opalescent or acquire more than a faint brownish tint when exposed to diffuse daylight for six hours. This is a U. S. P. test for organic impurities, amylic alcohol, aldehyde, etc. The imported alcohols are not up to standard at all times by this test.

9. Into a test tube which has been rinsed with the alcohol, pour 5 c.c. of sulphuric acid, then layer the acid with an equal amount of the alcohol. The appearance of a red zone after stand-

ing for four hours or longer indicates the presence of molasses alcohol. Neither domestic nor foreign alcohols respond to this test.

10. Pass hydrogen sulphide gas through 20 c.c. of the alcohol for from two to five minutes, then add a few drops of ammonia and allow to stand for four hours. The alcohol should remain colorless. If it becomes yellowish or brownish, the presence of traces of metal, extractives or tannin is indicated. By this test many of the samples of imported alcohol are shown not to be up to standard.

11. To 10 c.c. of the alcohol add 1 c.c. of a solution of potassium permanganate (1-1000). Allow to stand twenty minutes, the development of a yellowish or brownish color indicates the presence of aldehyde. Many of the imported alcohols show this impurity in more than traces. Our redistilled alcohol does not show it.

12. To 5 c.c. of the alcohol add two drops of a one per cent aqueous solution of furfurol, underlay this, kept cold in a stream of water, with 5 c.c. of sulphuric acid. The formation of a colored zone, gradually becoming pink, shows the presence of amylic alcohol. This is an exceedingly delicate test and by making a standard solution of amylic alcohol the proportion

can be approximated. Kahlbaum's alcohol, as we have found, often shows appreciable traces of amylic alcohol by this test.

13. To 10 c.c. of the alcohol add ten drops of colorless analin, then three drops of hydrochloric acid. No coloration should develop within five minutes. This is a test for furfurol, which we have not found in either domestic or imported alcohols.

14. Evaporate 100 c.c. of the alcohol to dryness, extract the residue with from 3 c.c. to 5 c.c. of salt solution and inject this intravenously into a guinea-pig of from 200 g. to 300 g. weight. With our redistilled alcohol there is no effect, while with Kahlbaum's the animal is often thrown into convulsions which may terminate fatally. What this residue contains, I have not been able to determine. It is granular and sometimes contains a few needle shaped crystals.

From my experience, I have found it better to use alcohol prepared in my own laboratory from the commercial ninety-five per cent article, by redistillation with caustic lime, than to depend upon the imported product.

PART V.

TISSUE CELLULAR PROTEIN POISONS.

Cummings and Chambers* have prepared the protein poison from various organs and tissues and have tested its effects upon both homologous and heterologous animals. They describe their methods of preparing the tissue as follows: Tissues including muscle, brain, heart, lungs, liver, pancreas, and kidney were obtained from the dog, goat, ox, and rabbit. Because the protein poison can be obtained from blood constituents it was necessary to remove these before clotting occurred, thus securing organs free from water soluble protein. The following procedure was carried out to gain this result with the dog, goat, and rabbit: The animal was first anesthetized, then a glass cannula was introduced into the abdominal aorta; the jugulars were then cut and water was forced under mild pressure into the circulatory system. Several liters of wash water were used in each case, the washing being continued until

*Cummings and Chambers: Jour. Lab. and Clin. Med., i, 428.

the fluid running from the jugulars had lost its red tinge. The ox organs were removed immediately after death, and each was washed free from blood by forcing water through its circulatory system. The organs were cut into half-inch cubes and washed in water with frequent changes; the final wash water gave none of the tests for protein. The tissues were then dehydrated in 80 per cent and 100 per cent alcohol each for twenty-four hours. They were then dried and powdered with a fine meat grinder.

The tissue thus prepared was split into poisonous and nonpoisonous parts by a 2 per cent solution of sodium hydroxide in absolute alcohol. The poisonous products were tested out on animals. Conclusions are stated as follows:

1. Vaughan's protein poison can be prepared from tissue cells of the exsanguinated organs of multicellular animals.

2. The tissue cellular protein poisons are not only toxic for heterologous species, but also for homologous species.

3. The M.L.D. of the protein poisons—here reported—for the guinea-pig and the rabbit is in proportion to their relative body weights when given by the intraperitoneal method of injection; when given intravenously, however, it

is, in proportion to body weight, twenty-five (25) times more toxic for the guinea-pig than the rabbit.

4. Tissue cellular protein poison hastens the clotting of the blood of the guinea-pig, rabbit, and dog in vivo. The protein poison prepared from casein differs from these in that it either retards or prevents entirely the clotting of dog's blood.

5. Witte's peptone does not prevent the clotting of rabbit's blood in vitro.

6. The in vitro experiments here reported show that all the protein poisons tested inhibit the clotting of blood from the guinea-pig, rabbit and dog, in certain percentages.

THE COLOR REACTIONS OF PROTEINS AND THEIR SPLIT PRODUCTS.

Emerson and Chambers* have made a comparative study of the protein color reactions on the original proteins and their poisonous and nonpoisonous split products. These studies give some conception of the lines along which the cleavage of the protein develops. The conclusions are stated as follows:

1. The proteins and their split products, the

*Emerson and Chambers: Jour. Lab. and Clin. Med., i, 692.

protein poisons and the residues, all give the biuret reaction. The residues will not give the reaction in dilutions much greater than 1-100. This indicates that the proteins and their split products contain an acid amide group and other substituted amide groups attached to neighboring carbon atoms, and that the end products have not been deamidized in cleaving.

2. Gies' biuret reagent gives similar results.

3. The proteins and their split products all give the xanthoproteic reaction, and the poisons, in greater dilutions than the proteins or the residues, indicating that they all contain benzene nuclei and that in cleaving, these tend to concentrate in the poison.

4. The residues do not give Millon's reaction and the poisons give it in greater dilutions than the proteins, indicating that all the mono-hydroxy-benzene nuclei (tyrosine) are cleaved off into the poison. It is interesting to note the strong reaction given by the colon cell substance and by the poison.

5. The residues do not give Bardach's reaction and all soluble proteins are said to give it. The residues are alkaline and soluble.

6. The poisons do not give the Molisch reaction and the residues give a stronger Molisch than the proteins, indicating that the carbohy-

drate groups are cleaved off into the residues. Casein gives a very weak Molisch reaction, and casein residue a strong reaction; this may be due to tryptophane, but we think not, as the casein residue gives only a weak tryptophane reaction. Casein residue does not reduce Fehling's solution even after boiling with dilute hydrochloric acid. Part of the residue is insoluble in 10 per cent alkali and this insoluble part gives the Molisch reaction and also tests for nitrogen.

7. The proteins and their split products all give the Adamkiewicz reaction indicating that they all contain the tryptophane group. Solutions of tyrosine give pinkish violet colors which are similar to the tryptophane reactions in dilute solutions.

8. The protein poisons do not give good Hopkins-Cole reactions when performed in the regular way. The proteins and residues do. They all give good positive reactions when furfural is added. The protein poisons probably give negative reactions because they contain no carbohydrate.

9. Benedict's modification of Hopkins-Cole reagent gives similar results both with and without furfural.

10. The Acree Rosenheim formaldehyde test

gives similar results to those obtained with the Hopkins-Cole reagent, both with and without furfural.

11. The proteins and the residues give positive results with the benzaldehyde test (Reichl's reaction). The poisons give negative reactions and the addition of furfural has no effect.

THE NINHYDRIN REACTIONS.

The same investigators have made a study of the ninhydrin test as applied to the original protein and the split products. This reaction has become prominent on account of its delicacy and because of its use in the Abderhalden test.

The conclusions in this study show that the poisonous split product gives this reaction in the same dilution as the original protein, while the nonpoisonous part fails to respond even in highly concentrated solution. Emerson and Chambers state their results as follows:

1. Vaughan's protein poisons in dilutions up to 1-10,000 give the ninhydrin reaction.

2. The proteins from which the poisons are obtained will also give the ninhydrin reactions in dilutions up to 1-10,000.

3. The cell residue in dilutions of 1-100 does not give the ninhydrin reaction.

4. Dilute acids and alkalis interfere with this reaction.

5. Sodium chloride interferes very slightly with the reaction.

6. Prolonging the time of heating makes the reaction very much more delicate.

THE PROTEIN SKIN REACTION.

Much use has been made in recent years of certain skin reactions for diagnostic purposes. Such is the von Pirquet test in tuberculosis. I have long held that this is a protein reaction. The tubercular individual elaborates in his body cells a ferment which splits up tuberculin and sets the protein poison free, and the reaction is due to the local effects of the liberated protein poison on the epithelial cells. This explanation is confirmed by recent observations. A man claimed that he could not eat even a small amount of any food containing nutmeg without soon becoming sick and developing urticarial rash. A bit of nutmeg was crushed in a mortar in a few drops of sterile salt solution and a drop of this suspension placed on the forearm and scratched in with a von Pirquet scarafier. Within a few minutes a large wheal or hive developed about the point. Similar tests have been made with similar results on those sensitive to pep-

tone, egg white, etc. The explanation is further confirmed by the demonstration that the protein poison liberated by chemical agents in vitro produces a like effect upon all cells. By this simple procedure the sensitiveness of persons to certain proteins may be demonstrated and the degree roughly approximated. Possibly sensitiveness to horse serum might be tested in this way preliminary to the administration of diphtheria antitoxin, but I have not known of its being tried.

INDEX

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